11.—The development of the gametophyte and young sporophyte of Ecklonia radiata (C.Ag.) J.Ag. (Laminariales)

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Abstract

Gametophytes and young sporophytes of *Ecklonia radiata* have been grown under controlled environmental conditions, and their structure and development is described. Both the male and female gametophytes are very reduced compared with those of most other members of the order. A periodic alternation of cell division and elongation was observed during the development of male gametophytes.

Introduction

Ecklonia radiata (C.Ag.) J.Ag. is the only representative of the Laminariales found in Western Australian waters. It occurs throughout the waters of southern Australia, Lord Howe Island and New Zealand (Lindauer *et al.*, 1961). The life history of this plant has not hitherto been studied, and it is the purpose of this paper to record the development of its gametophyte and young sporophyte.

Materials and methods

Mature sporophytes were collected from reefs in the shallow sub-littoral zone at Cottesloe, during the period March to July inclusive. Fertile areas of the thallus were located by examining sections under the microscope. These areas were thoroughly inspected under a dissecting microscope, and only segments which appeared free of epiphytes were used in the following work.

Cultures were prepared in the manner described by Papenfuss (1942). Suitable material was kept out of water for several hours to help promote spore release. Pieces of thallus, about 6 cm square, were then immersed in filtered sea water and brushed with a camelhair brush to remove excess mucilage. They were then placed for several minutes in petri dishes of filtered sea water containing microscope slides. Zoospores were released during this period, and a further 12-24 hours were allowed for them to become attached to the slides. The slides were then removed, and filtered sea water was run over them to wash away as many diatoms and protozoa as possible.

The slides were then placed in 14 cm-diameter petri dishes (5 slides per dish) containing 100 ml of nutrient solution. This consisted of $NaNO_3$ 0.1g; Na_2HPO_4 0.02g; distilled water 50 ml; filtered sea water 1,000 ml (Schreiber, 1930). Five ml. of ethylene diamine tetraacetic acid (E.D.T.A.), 3.7 g/l, was included as a chelating agent. The medium was not changed during the period of growth. The cultures were placed in growth cabinets under a light intensity in the region of 1500 foot candles provided from a mixture of fluorescent tubes and incandescent globes. The daylength was 16 hours, the temperature 22°C.

Slides were removed and examined from time to time, and returned to the nutrient solution immediately after examination. In order to gain an estimate of variation between individuals, 20 randomly selected plants were examined. Illustrations were drawn from living material, but observations on nuclei were made from plants stained with Harris' Haematoxylin (Johansen 1940).

Observations

The gametophyte

After a period of motility ranging from minutes to several hours, zoospores come to rest on the substratum, assume a rounded form and secrete a wall. Germination begins almost immediately, and in six-hour cultures a number of spores have recognizable germ tubes. There appear to be two main patterns of germination.

Firstly, the end of the germ tube swells, giving the "dumbbell" appearance described in related plants (Papenfuss 1942; Kanda 1941). In some cases the entire protoplast migrates into the swollen portion (Fig. 1a), and a cross wall may or may not form. Alternatively, part of the protoplast may remain in the spore case. If a cross wall does form, the cytoplasm in the spore degenerates, but if not the spore appears to remain living.

Secondly, the germ tube remains tubular (Figs. 1b,c), and a cross wall may or may not form. If no wall is formed, part of the protoplast may remain within the spore case.

By the third day, all plants exist in one of these conditions, after which further vegetative growth occurs over a period of seven days. The subsequent growth can be divided into several distinct phases (Fig. 2). From day 3 to 5 there is a period during which the single-celled plant elongates, and between days 5 and 7 an increase in cell number occurs. Cell extension again predominates between day 7 and 9, following which all further growth is, in the main, attributable to an increase in cell number.

Extension of the plant may occur in the plane containing the spore case and germ tube (Fig. 1a), or at various angles to this (Figs. 1f,g). In the latter case, the first cell may extend in one direction (Fig. 1f) or in opposite directions (Fig. 1g).

Male and female plants may be clearly distinguished after 10 days (Figs. 1f,i). The female plants are invariably more heavily pigmented

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than the males, and the cells of the females are approximately twice the length and breadth of the cells of the males. At maturity male plants are on the average 1.4 times as long as female plants. This difference is due to the fact that each male plant consists of two to five cells, while 90 to 95 per cent of female plants comprise only one cell (Table 1). It seems certain that male and female plants begin to differ in development between days 5 and 7, for at day 7, 50 per cent of plants were found to have undergone a single nuclear and cell division. This division was almost certainly restricted to male plants.

TABLE 1

Length and cell number of eleven-day-old male and female gametophytes of Ecklonia radiata. *Standard errors are given in brackets.*

	Male Gametophyte	*Female Gametophyte*
Mean length (microns)	34.3 (3.2)	23.7 (2.3)
Mean cell number	3.2 (0.4)	1.1 (0.1)

Reproductive structures begin to differentiate by days 10 to 11, when vegetative growth ceases. In male plants antheridia appear as lateral or terminal projections which are eventually separated from the parent cell by a wall. More than one antheridium may be produced from one cell, and up to nine have been observed on one plant. It is possible that more may form during prolonged culture. The antheridium is almost colourless, and the entire protoplast forms a single spermatozoid, which escapes through a terminal pore (Fig. 1k). The spermatozoids may be pyriform or spherical, and of the order of 5 microns in length or diameter respectively. They are biflagellate, and do not have eye spots.

The single cell of a typical female plant develops into an oogonium, in which the protoplast becomes a single ovum with a densely granular appearance and strong pigmentation. Many plants release ova by day 14 (Fig. 1). During the release a marked internal orienta-

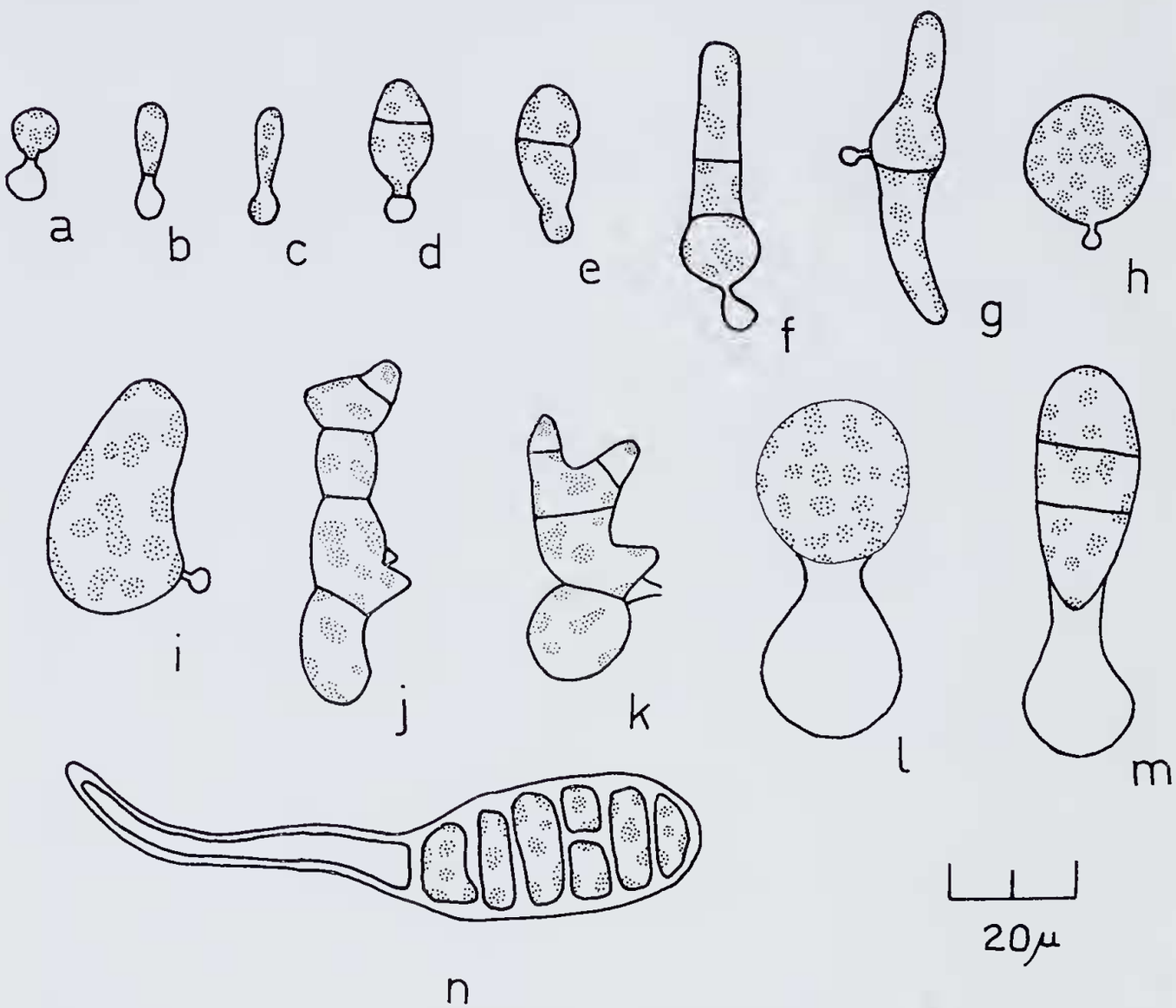

Fig. 1.—Stages in the development of the gametophyte and young sporophyte of *Ecklonia radiata*. All figures are camera lucida drawings of plants grown in nutrient solution. a, b, c, gametophytes, 3 days old. d, e, gametophytes, 6 days old. f, g, male gametophytes, 10 days old. h, i, female gametophytes, 10 days old. j, male gametophyte, 11 days old, showing developing antheridia. k, male gametophyte, 14 days old, one antheridium empty. l, female gametophyte, 14 days old, egg extruded from oogonium. m, young sporophyte. n, young sporophyte, showing rhizoid development.

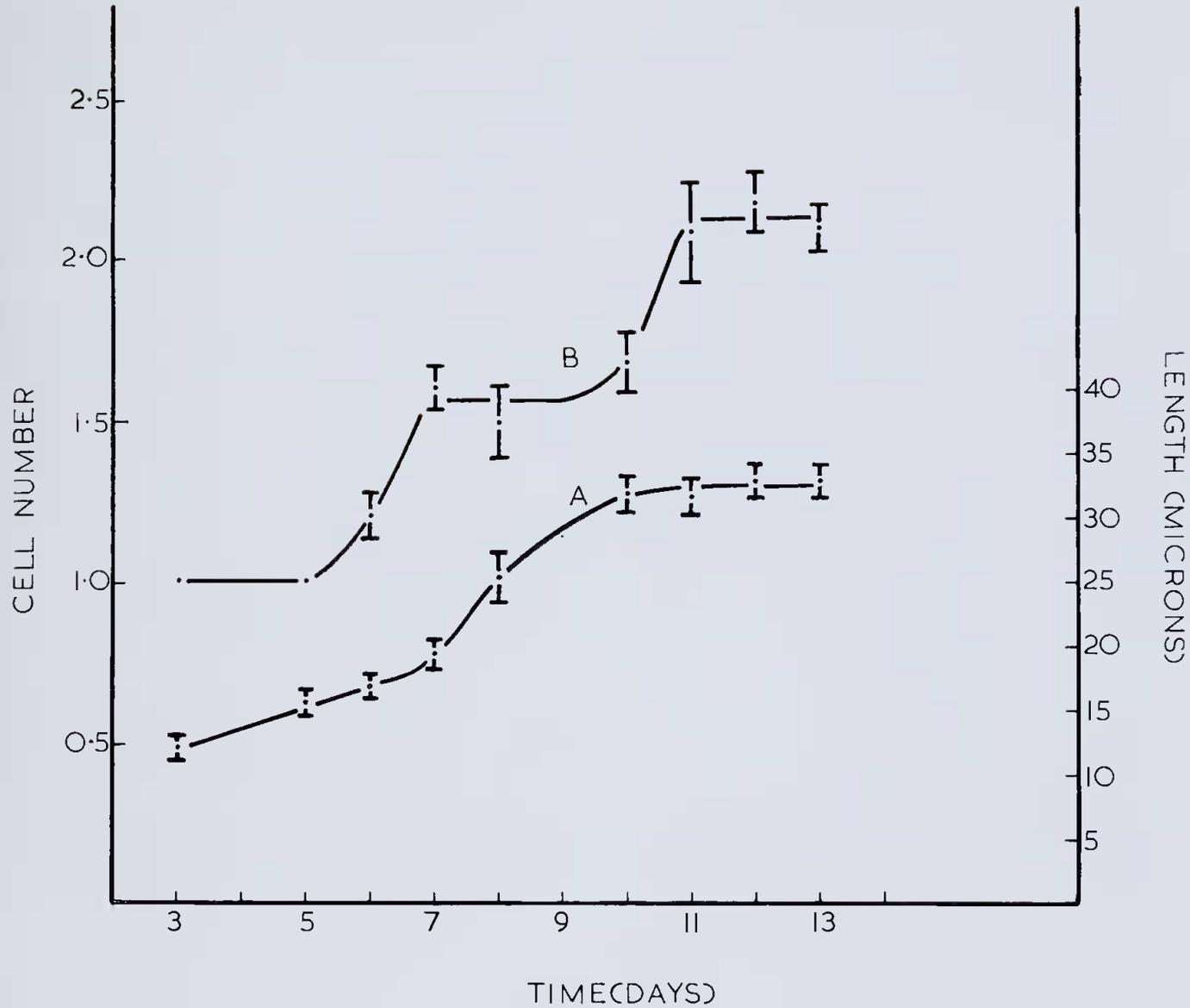

Fig. 2.—Length (A) and cell number (B) of *Ecklonia radiata* gametophytes. Vertical bars indicate standard errors.

tion of cell inclusions is noticeable. Fritsch (1945) suggested that similar phenomena in related plants may be due to strong internal pressures playing a role in dehiscence. The ovum, which is generally spherical but which may occasionally be spheroidal, remains attached to the lip of the oogonial case during fertilization and early development of the sporophyte.

Despite the constant culture conditions, development of gametophytes was found to be much slower in winter than in summer. This has also been observed for *Ecklonia maxima* by Papenfuss (1942). Detailed observations were not made beyond day 10 in winter, when plants were at a similar stage of development as those in 5 day-old summer cultures.

The young sporophyte

Development of the young sporophyte commences soon after fertilization, and while still attached to the oogonial lip. The zygotic cell commences to elongate before transverse divisions occur (Fig.1m). Between 4 to 10 cells are usually formed in this manner before longitudinal septation begins. All cells, with the exception of the basal cell, undergo longitudinal division. By the time the plant is 4 to 8 cells long, the basal cell has become elongate, and forms the first rhizoid (Fig.1n). The chromatophores degenerate rapidly. This situation was observed in a number of 12 to 15 day-old plants. Development was not followed beyond this stage.

Discussion

The development of *Ecklonia radiata* gametophytes and young sporophytes is in general similar to that described for other members of the genus *Ecklonia* (Papenfuss 1942; Kanda 1941), and the order Laminariales (Fritsch 1945), but there are differences in detail. It would seem that in most other species of the order, germination of the zoospores involves either the migration of all of the protoplasm from the spore, or the degeneration of any remaining in the spore. An exception is *Alaria*

esculenta, where the spores have been reported by Sauvageau (1916) to retain some cytoplasm and to germinate again to form a second gametophyte. In contrast, in *Ecklonia radiata* cytoplasm may remain in the spore, which continues as a living part of the plant.

Both male and female plants are extremely reduced, with most female plants having no vegetative filaments. This is a condition recorded for *Ecklonia cava* and *Ecklonia stolonifera* (Kanda 1941), though not for *Ecklonia maxima* (Papenfuss 1942). Unicellular female plants and reduced male plants are also reported in several other laminarian genera (Fritsch 1945; Evans 1965). The form and size of gametophytes have been shown to be affected to some extent by temperature, light intensity and nutrient solution (Fritsch 1945; Kanda 1941; Evans 1965), and so the possibility remains that less reduced gametophytes could develop under cultural conditions other than those used here.

Correlated with the reduced nature of the gametophytes is the unusually early stage at which the sexes begin to differ in development, and the early formation of reproductive organs. Kanda (1941) reports a similar situation to exist with *Ecklonia cava* and *Ecklonia stolonifera*, while Williams (1921) and Evans (1965) have made similar observations for *Laminaria* species. Gametophytes of most other Laminariales are considerably larger, and both the visible distinction of the sexes and the development of reproductive organs occurs much later in development; for example, this is the case with *Ecklonia maxima* (Papenfuss 1942).

Evans (1965) suggested the possibility that there is a rhythm of cell divisions during gametophyte growth. This is clearly the case with *Ecklonia radiata*, and similar methods of analysis may prove it to be widespread among laminarian gametophytes. It would be of interest to study the periodicity of the divisions in relation to variation in the environmental conditions.

Kanda (1941) has reported that the basal cell of the young sporophyte of *Ecklonia cava* elongates, but is not involved in rhizoid formation. In this respect it is clear that *Ecklonia radiata* more closely resembles *Ecklonia maxima*, for Papenfuss (1942) found that its basal cell becomes rhizoidal.

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